Pak. J. Bot., 44(3): 1159-1165, 2012.

AMINO ACIDS COMPOSITION OF MYCELIAL PROTEIN OF *ENICILLIUM EXPANSUM* GROWN IN ACID TREATED RICE HUSK MINERAL MEDIUM

M. YAKOUB KHAN, M. UMAR DAHOT*, S.HABIB AHMED NAQVI*

*Institute of Biochemistry and *Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan.*

Abstract

The aim of the present study was to analyze the amino acids composition of single cell protein of *Penicillium expansum*. Mycelial biomass was produced when fungus was grown in 0.6N H_2SO_4 pretreated rice husk mineral medium incorporated with 0.5% and 1% of nitrogen sources like potassium nitrate, sodium nitrate, ammonium nitrate, peptone, yeast extract, urea, corn steep liquor and ammonium sulphate. It was observed that the growth rate of *Penicillium expansum* increased with 0.5% sodium nitrate produces 1.390 ± 0.084g/l of mycelial biomass. In the subsequent experiment, fermentation medium was supplemented with 0.5% and 1.0% different sugars (sucrose, glucose, fructose, maltose, galactose, lactose, carboxymethyl-cellulose, starch, mannose, and molasses) at pH 6.0 for 240 hours at 35 ± 2°C in a fermenter. The highest amount of mycelial biomass (5.107 ± 0.169g/l) was obtained with 1% sucrose and followed by 4.953 ± 0.17g/l, 4.808 ± 0.14g/l and 4.844 ± 0.10g/l mycelial biomass using glucose, maltose and galactose, respectively. The mycelial biomass of *Penicillium expansum* contains essential and non essential amino acids like phospho-serine, serine, valine, aspartic acid, threonine, glutamic acid, glycine, isoleucine, leucine, phenylalanine, alo-lysine, halo-lysine, lysine and arginine. The glutamic acid (3355.0 ± 19.798 µmol/g mycelia) and proline (785.0 ± 9.899 µmol/g mycelia) were found in higher concentration than other amino acids produced by *Penicillium expansum* grown on rice husk supplemented with lactose.

Introduction

The developing countries facing the problems of nutrition and they have excess of agricultural wastes materials, which are rich in carbohydrates. These wastes materials can be utilized after treatment as a carbon source with mineral medium in fermentation processes for the production of value added goods including antibiotics, vitamins, vaccines and single cell protein etc, which will solve the problems of food and feed crisis (Bhattacharjee, 1970). The FAO predicted an annual protein deficit of 10 million tons in 1980, which increased to 22 million tons in 2000 and this shortage can be increased to 45 million tons by 2020. The microbial mycelial proteins can be used for the supplementation of available food and feed for human, poultry and animal consumption. The conventional sources of proteins are relatively more expensive and there is need to search alternative sources which fulfill the requirements of protein demand. The developing countries having plentiful supply of agricultural waste substrate which can be utilize for the production of single cell protein (Moo-Young, 1977). Single cell protein is the most useful for human and animals nutrition because it consists of all essential and non-essential amino acids in correct ratio, which is generally accepted as more or less ideal after its comparison with other food and feeds (Algur & Gokalp, 1991a; Bernstein *et al.,* 1977; Christias *et al*., 1975; Ginterova & Lazarova 1987).

Single cell protein in various forms has attracted particular interest because of its amenability to controlled intensive cultivation and nutritional conditions. Among these conditions pH, temperature, aeration rate, size of inoculum, carbon source, nitrogen source and the use of minerals in different concentrations in fermentation medium are of considerable importance and significant effects (Oshoma & Ikenemomeh, 2005; Sanna & Sabry, 1991; Zabrodskii, 1972). The production of single cell protein as a food or feed supplementation has received much attention in recent years due to acute shortage of grains and fish meal in many countries (Daghir & Baki, 1977; Gow *et al.,* 1975; Gregory *et al.,* 1976; Onol & Yalcm 1995; Sehu *et al.,* 1977; Scrimshaw, 1968; Rubbani *et al*., 2011). However, for human and animal nutrition most microbial proteins are low in methionine or tryptophan and both amino acids are essential for animals and humans (Anderson & Jacson, 1958a, Anderson *et al.,* 1958b, Nelson *et al.,* 1960). Okanishi & Gregory (1970) have been successful in overcoming this problem by using high concentration of sulphur in the growth medium of isolated mutant of *Candida tropicalis* and dry biomass of single cell protein contains 41.0% more methionine than parent isolate. Single cell protein can be used as a protein source for poultry feed, livestock and even for human consumptions (Pacheco *et al*., 1997).

Pakistan is a developing country facing the financial crises due to flood and the war against terrorism. The prices of different utility things including the meat rates increases and the shortage of meat also produced in the country due to the flood. The poultry feed industry also facing the shortage of quality feed due to the high rates of different grains. The possible alternate is to produce single cell protein by microorganism using agro-industrial wastes as carbon and nitrogen source through fermentation process. Pakistan is an agricultural country producing millions of tones cellulosic biomass such as sugarcane, rice husk, wheat and cereal husk and straw (Anon., 1988). These agricultural wastes mainly consists cellulose, hemicellulose and lignin (Han, 1978). Mostly this waste is currently disposed by open burning in the field, which produces air pollution. In present study, the rice husk was used as a carbon and nitrogen source for the growth of *Penicillium expansum* and single cell protein production. The single cell protein was supplemented with available feed. After feeding trial, the growth and weight gained by chicks was observed. The chicks produced through single cell protein feed can be sold at relatively low prices with better nutritional quality than convectional ones (Oloyede *et al.,* 2007).

Materials and Methods

Substrate: Rice husk of Iri-Pak 6 was used throughout the study and was collected from Larkana Rice Mill, District Larkana, Sindh.

Microorganism: *Penicillium expansum* strain CMI 39761 was obtained from the Department of Botany, University of Glasgow, U.K. and was used in this study. The stock culture was maintained on agar slant, containing (g/L) Dextrose: 20; peptone 10 and agar 20. The ingredients were thoroughly mixed and kept in culture tubes and sterilized at 1.5 kg/cm^2 for 20 minutes. The sterilized slants were inoculated with *Penicillium expansum* and incubated at 27°C to obtain luxuriant growth.

Methods

Preparation and degradation of rice husk with acid treatment: Rice husk was pretreated with acids (H_2SO_4 / $HClO_4$) as reported earlier (Yakoub *et al*., 1992).

Culture medium: Culture medium was used for the growth of *Penicillium expansum* as reported by (Burrel *et al.,* 1966), without altering chemical composition.

Cultivation condition: The required amounts of minerals were added in 100 ml of rice husk hydrolysate in conical flask which were covered with cotton plug and autoclaved at 1.5kg/cm^2 for 20 minutes. The sterilized media, cooled at room temperature were inoculated with 2.0 ml spores (50 x 10^6 /ml) of *Penicillium expansum* .The sugars were sterilized separately and added aseptically. These flasks were kept in orbital cooled shaking incubator (200 rev/min) at 26°C. Culture broth was separated from mycelia after 48 hours by filtration.

Fermentation parameters: Mycelial biomass of *Penicillium expansum* was produced when fungus was grown in 0.6N H_2SO_4 pretreated rice husk mineral medium supplemented with 1% different sugars (glucose, galactose, fructose, sucrose, lactose and starch) at pH 6.0 for 240 hours at 35°C in a 5L fermenter (Gallen Kamp).

Determination of mycelial biomass: The quantity of the mycelial biomass was noted after washing with distilled water and drying at 105-110°C.

Determination of protein: Nitrogen content of mycelial biomass was determined by Kjeldahl method (Harold, 1950). The crude protein values were obtained by multiplying the nitrogen content by 6.25.

Amino acids analysis by thin layer chromatography: Amino acids were identified from single cell protein biomass by two dimensional thin layer chromatography using activated plate of silica gel G 60 (E.Merck) of 0.25 mm thickness. The eluent used was butanol: acetic acid: water (4:1:1 v/v/v) and phenol water (4:1 v/v) and ninhydrin as developer.

Amino acids analysis by amino acid analyzer: The amino acids analysis of the single cell protein biomass of *Penicillium expansum* was carried out by automatic amino acid analyzer (Hitachi 835 with 150 X 2.6 mm C-18 column) after hydrolysis of the sample protein in 6 N HCl for 24 hours (Algur *et al*., 1991b)

Results and Discussion

The results obtained with the effect of 0.5% and 1% of nitrogen sources tested along with 0.6N H_2SO_4 pretreated rice husk on the production of single cell protein by *Penicillium expansum* in Figs. 1 and 2, respectively. The mean of single cell protein obtained were 0.579 ± 0.07, 1.390 ± 0.084, 1.225 ± 0.127, 0.587 ± 0.096, 0.424 ± 0.021, 0.405 ± 0.028, 0.596 ± 0.063 and 0.610 ± 0.028g/l with 0.5% potassium nitrate, sodium nitrate, ammonium nitrate, peptone, yeast extract, urea, corn steep liquor and ammonium sulphate, respectively. The maximum mycelial protein (1.390 ± 0.084g/l) was obtained with the use of sodium nitrate as a nitrogen source. Whereas, the mean of single cell protein obtained were 1.157 ± 0.048, 0.602 ± 0.028, 1.271 ± 0.069, 0.591 ± 0.029, 0.446 ± 0.05, 0.608 ± 0.027, 0.608 ± 0.013 and 1.102 ± 0.104g/l with 1% potassium nitrate, sodium nitrate, ammonium nitrate, peptone, yeast extract, urea, corn steep liquor and ammonium sulphate, respectively as nitrogen sources. The maximum yield (1.271 ± 0.069 g/l) of single cell protein among the nitrogen source was with the use of ammonium nitrate. The inorganic salts of nitrogen sources produce greater amount of single cell protein than complex organic nitrogen sources. It has been reported in the literature that requirement of specific nitrogen source is different for different microorganisms. For instance *Penicillium cyclopium* produces higher amount of single cell protein when urea or ammonium hydroxide were used as a nitrogen source (Kim *et al.,* 1981). Urea, ammonium nitrate, sodium nitrate, potassium nitrate were found suitable for the maximum production of single cell protein by *Aspergillus fumigatus* when cassava was used as carbon source (Reade & Gregory *et al.,* 1975). Higher amount of single cell protein was produced by *Aspergillus terreus* when ammonium sulphate was used as nitrogen source (Garg & Neelakantan *et al.,* 1982).

The effect of 0.5% and 1% of carbon sources along with 0.6N H_2SO_4 pretreated rice husk on the production of Single cell protein by *Penicillium expansum* is presented in Figs. 3 and 4, respectively. The mean Single cell protein obtained were 3.596 ± 0.03, 3.057 ± 0.197, 2.304 ± 0.134, 4.016 ± 0.178, 3.896 ± 0.148, 3.252 ± 0.183, 1.485 ± 0.197, 3.890 ± 0.290, 2.974 ± 0.207 and 2.904 ± 0.155g/l with 0.5% of sucrose glucose, fructose, maltose, galactose, lactose, carboxymethyl-cellulose, starch, mannose and molasses, respectively. The favorable substrate for the maximal production of single cell protein (4.016 ± 0.178 g/l) was with 0.5% maltose. Furthermore, the Single cell protein obtained were 5.107 ± 0.169, 4.953 ± 0.17, 4.022 ± 0.12, 4.808 ± 0.14, 4.844 ± 0.107, 2.988 ± 0.13, 1.287 ± 0.14, 4.448 ± 0.07, 3.125 ± 0.16 and 4.377 ± 0.07 with 1% of sucrose glucose, fructose, maltose, galactose, lactose, carboxymethyl-cellulose, starch, mannose and molasses, respectively. The higher yields (5.107 ± 0.169g/l) of Single cell protein obtained with 1% sucrose among the other sugars were used as carbon sources. During the course of growth the rise of pH was observed with 0.5 % carbon sources. It might be due to exhaustion of carbon source which inhibits the growth of *Penicillium expansum*. It has been reported that *Kluyveromyces fragilis* produces higher amount of single cell protein in lactose medium than glucose used as substrate (Garibay *et al.,* 1987).

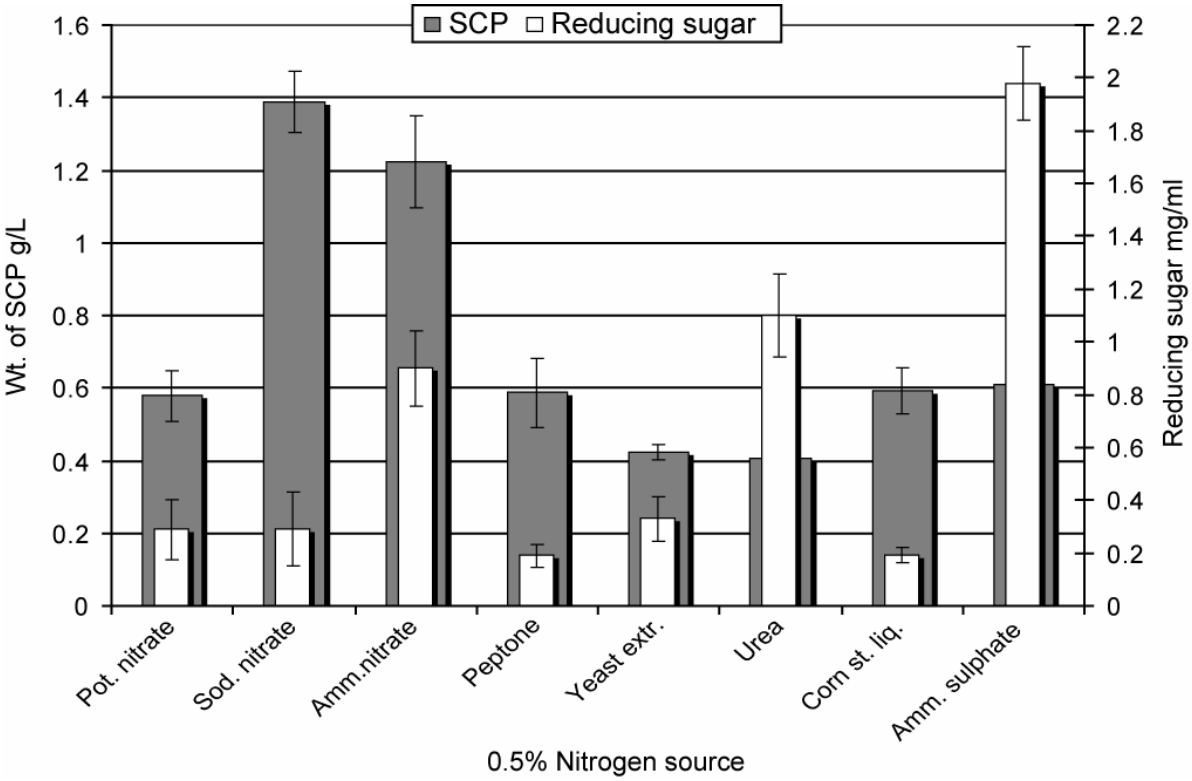

Fig. 1. Effect of 0.5% nitrogen source on the production of amino acids from single cell protein by *Penicillium expansum*, grown on 0.6N H_2SO_4 pretreated rice husk mineral medium at 35±^oC for 240 hours with initial pH 4.0. Results represent the mean of duplicate analysis and bar indicates ± standard deviation.

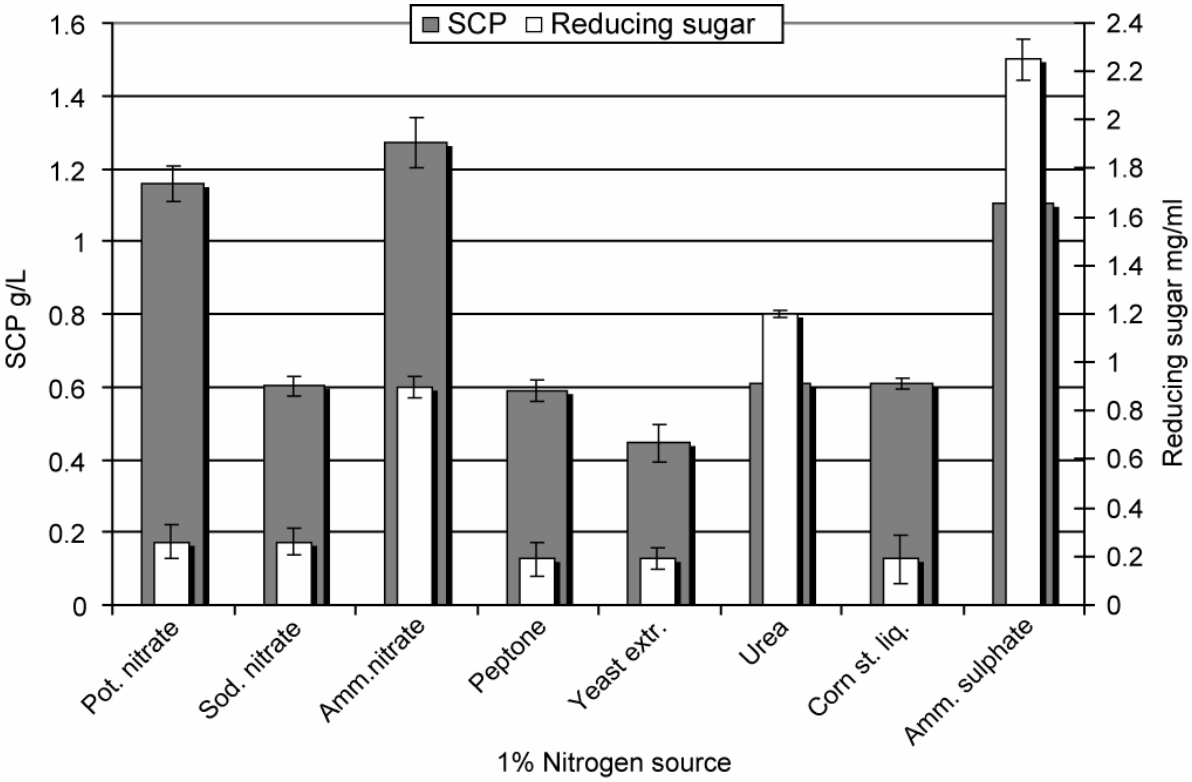

Fig. 2. Effect of 01% nitrogen source on the production of amino acids from single cell protein by *Penicillium expansum*, grown on 0.6N H_2SO_4 pretreated rice husk mineral medium at 35±^oC for 240 hours with initial pH 4.0. Results represent the mean of duplicate analysis and bar indicates ± standard deviation.

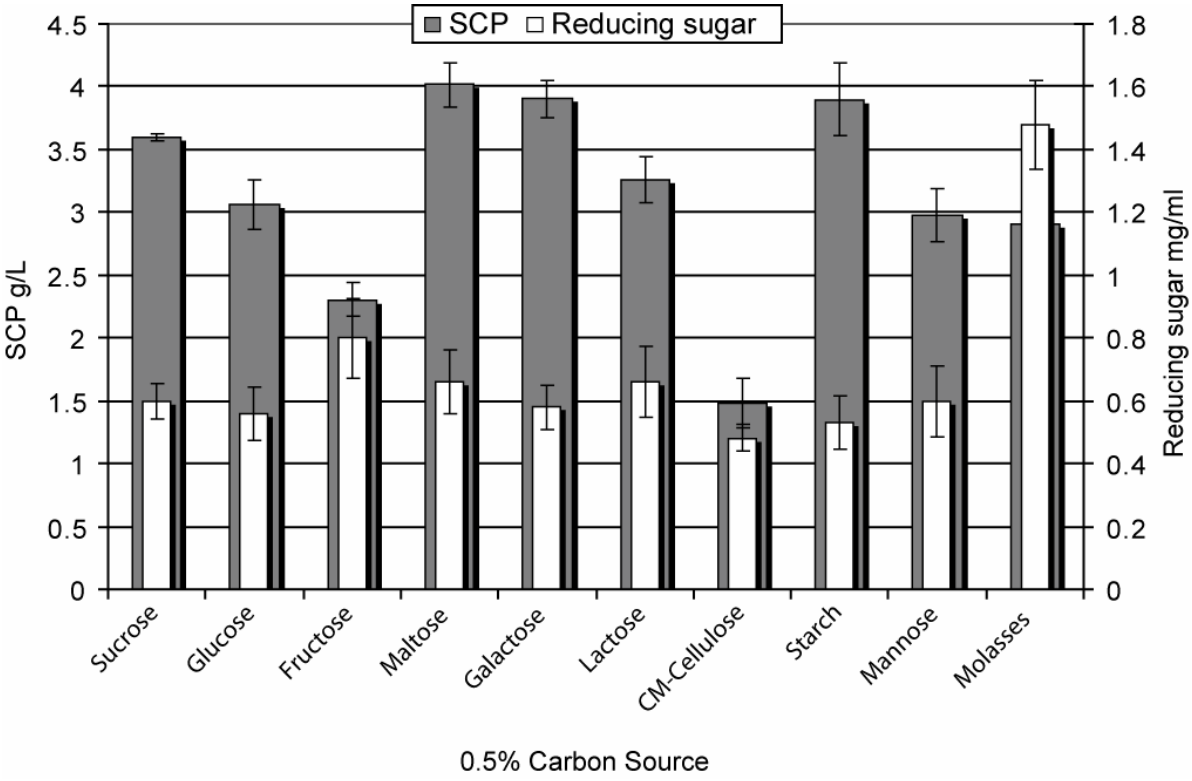

Fig. 3. Effect of 0.5% sugars as a carbon source on the production of amino acids from single cell protein by *Penicillium expansum* grown on 0.6N H_2SO_4 pretreated rice husk mineral medium at 35±^oC for 240 hours with initial pH-4.0. Results represent the mean of duplicate analysis and bar indicates ± standard deviation.

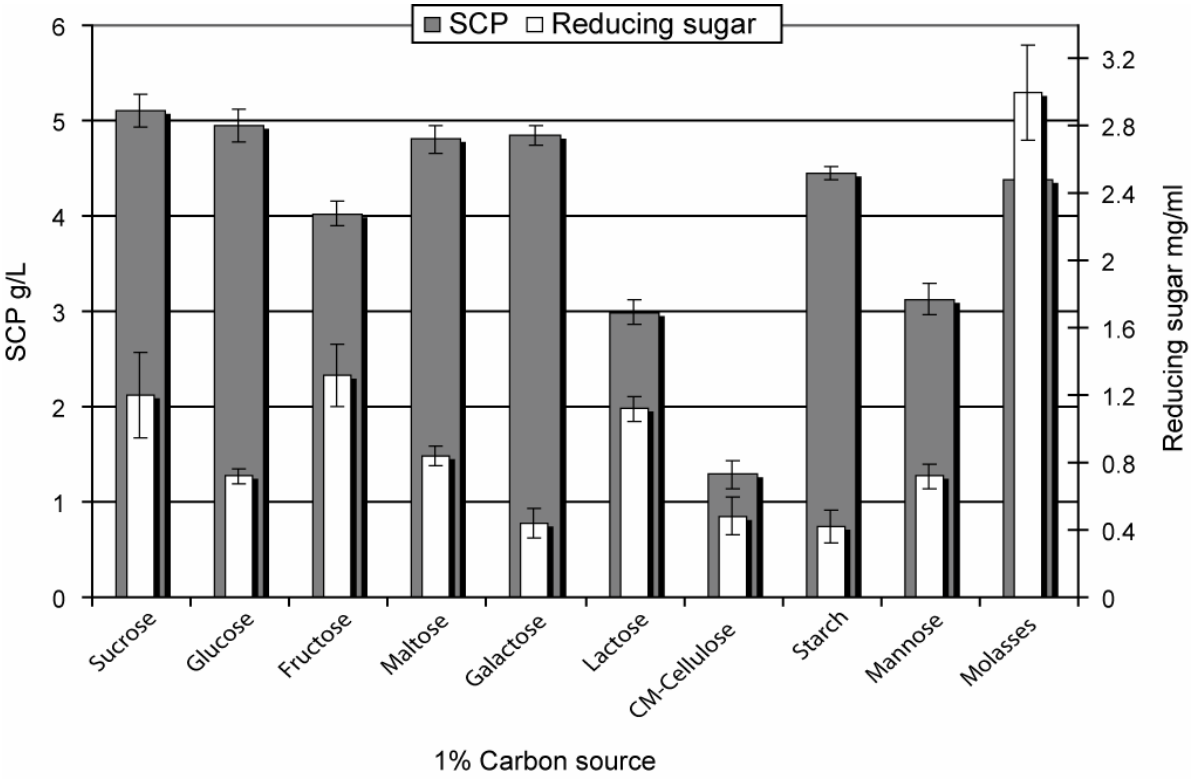

Fig. 4. Effect of 1% sugars as a carbon source on the production of amino acids from single cell protein by *Penicillium expansum*, grown on 0.6N H_2SO_4 pretreated rice husk mineral medium at 35±^oC for 240 hours, at initial pH-4.0. Results represent the mean of duplicate analysis and bar indicates ± standard deviation.

The proximate quantitative composition of amino acids detected by thin layer chromatography from single cell protein biomass is shown in Table 1. Almost all essential amino acids are present in 0.6N sulphuric or perchloric acid pretreated rice husk mineral medium. The data indicates that *Penicillium expansum* single cell protein contains similar amino acids as found in microbial proteins (Crawford & Mccy, 1973; Samuel & Zimmerman., 1977).

Table 2 demonstrates the quantitative composition of amino acids of single cell protein produced by *Penicillium expansum* grown on rice husk hydrolysate with and without sugars as carbon sources. The mycelial biomass of *Penicillium expansum* is rich in essential and non essential amino acids. The abundant amount of amino acids was obtained when rice husk mineral medium was supplemented with fructose, sucrose and lactose. The maximum yield of amino acids like phospho-serine, serine, valine and lysine in the concentration of 504.0 ± 9.899, 327.0 ± 14.142, 3124.0 ± 28.284 and 267.0 ± 12.727µmol/g mycelia are present in mycelial biomass, respectively with the use of rice husk supplemented with fructose. The concentration of aspartic acid, threonine, glutamic acid, glycine, isoleucine, leucine, phenylalanine, alo-lysine, halo-lysine, lysine and arginine were higher 812.5 ± 15.556, 494.5 ± 11.737, 3305.0 ± 14.566, 978.0 ± 14.142, 68.5 ± 13.152, 562.5 ± 14.707, 553.0 ± 12.727, 555.0 ± 7.071, 922.0 ± 11.313 and 539.5 ± 8.061µmol/g mycelia among the amino acids produced by *Penicillium expansum* grown on rice husk mineral medium supplemented with sucrose. The glutamic acid (3355.0 ± 19.798 µmol/g mycelia) and proline (785.0 ± 9.899 µmol/g mycelia) were found in higher concentration than other amino acids in Single cell protein of *Penicillium expansum* grown on rice husk supplemented with lactose. The absence of tryptophan, methionine and cysteine was probably due to their degradation during the acid hydrolysis of protein (Bressani *et al.,* 1987).

Table 1. Amino acids composition of *Penicillium expansum* single cell protein by thin layer chromatography.

Amino acids	Rf Values	$HClO_4$ Treated rice husk	H_2SO_4 Treated rice husk	H_2SO_4 Treated rice husk + Galactose
Arginine	0.300	+	+	+
Lysine	0.252	+	+	+
Cysteine	0.464	+	+	+
Leucine	0.469	+	+	+
Alanine	0.304	+	+	+
Histidine	0.404	-	-	+
Cystine	0.439	-	-	-
Serine	0.252	-	-	-
Asparagine	0.363	+	+	+
Phenylalanine	0.628	+	+	+
Valine	0.409	+	+	+
Glutamine	0.426	-	+	+
Tyrosine	0.400	+	+	+
Glutamic acid	0.076	+	+	+
Aspartic acid	0.100	+	+	+
Tryptophan	0.521	*	*	*
Threonine	0.279	+	+	+
Isoleucine	0.433	+	+	+
Methionine	0.525	+	+	+
Proline	0.542	+	+	+
Hydroxyproline	0.312	-	-	+
Glycine	0.406	-	-	+

(*) not determined, (-) Absent, (+) Present

The comparison of amino acids of *Penicillium expansum* single cell protein was comparable with fungi (such as *Pleurotus*; *Aspergillus niger*) and yeast (such as *Candida Ly 496*, *Fusarium oxysporum*) SCP as shown in Table 3. It is evident that *Penicillium expansum* does not suffer from any differences in individual or total amino acids content. However, it should be kept in mind that the biological availability of protein or bound amino acids depends on several factors which might be different in the case of filamentous fungi and yeast (Bernstein *et al.,* 1977). *Penicillium expansum* contains nearly all essential amino acids such as threonine, valine, isoleucine, leucine, phenylalanine, arginine and lysine in sufficient quantities. Single cell protein of *Penicillium expansum* contains higher amount of valine and lysine than *Aspergillus niger* (Christias *et al.,* 1975), *Pleurotus strain* (Ginterova *et al*., 1987) and *Fusarium oxysporum* (Christias *et al*., 1975). It is pointed out that comparison is only tentative, since the substrate of the medium used for the growth of these microorganisms for the production of single cell protein were different.

It is evident that single cell protein produced by *Penicillium expansum* supplemented with sucrose contains sufficient amount of essential and non essential amino acids which are quite favorable as compared with the ideal protein; therefore, the mycelial biomass by *Penicillium expansum* may be suitable for the supplementation in available food and feed for human, poultry and animal consumption.

Table 2. Production of amino acids from single cell protein of *Penicillium expansum* grown on 0.6N H_2SO_4 pretreated rice husk and rice husk contain 1% different sugars mineral medium at 35±˚C for 240 hours, at initial pH-4.0.

Amino acids µmol /g mycelia	Rice husk	Rice husk + glucose	Rice husk + galactose	Rice husk + fructose	Rice husk + Sucrose	Rice husk + lactose	Rice husk + starch
Phos-serine	-	288.0 ± 14.142	-	504.0 ± 9.899	252.4 ± 19.516	252.0 ± 11.313	279.9 ± 11.455
Aspartic acid	-	-	-	124.0 ± 12.727	812.5 ± 15.556	783.5 ± 12.727	585.5 ± 16.970
Threonine	177.0 ± 8.485	3307.0 ± 26.870	91.0 ± 2.828	364.0 ± 15.556	494.5 ± 11.737	468.5 ± 8.485	376.1 ± 9.899
Serine	142.0 ± 5.656	-	92.2 ± 5.939	327.0 ± 14.142	-	238.0 ± 16.970	396.6 ± 8.485
Glutamic acid	142.0 ± 4.242	1184.0 ± 28.284	197.0 ± 8.485	1677.0 ± 22.627	3305.5 ± 14.566	3355.0±19.798	2083.1 ± 13.435
Proline	6.4 ±1.555	-	9.6 ± 0.707	690.0 ± 16.970	646.0 ± 21.213	785.0 ± 9.899	547.7 ± 8.061
Glycine	758.0 ± 14.142	1915.0 ± 19.798	277.0 ± 5.656	733.0 ± 14.142	978.0 ± 14.142	730.0 ± 14.142	814.9 ± 9.899
Valine	1593.7 ± 42.85	-	531.0 ± 11.313	3124.0 ± 28.284	1093.5 ± 15.273	624.5 ± 5.656	1383.2 ± 12.727
Isoleucine	90.9 ± 7.071	567.0 ± 11.313	19.7 ± 0.424	197.0 ± 18.384	468.5 ± 13.152	370.0 ± 9.899	191.6 ± 5.656
Leucine	135.1 ± 11.172	1130.5 ± 13.576	33.0 ± 1.414	496.0 ± 11.313	1020.0 ± 15.556	744.0 ± 12.586	489.9 ± 6.929
Phenylalanine	59.2 ± 1.131	450.0 ± 14.142	22.5 ± 0.848	300.0 ± 8.485	562.5 ± 14.707	337.5 ± 12.445	249.9 ± 5.656
Alo-lysine	3.0 ± 0.707	676.0 ± 22.627	6.1 ± 0.565	276.0 ± 19.798	553.0 ± 12.727	535.0 ± 9.616	-
Halo-lysine	4.0 ± 0.282	678.0 ± 28.284	6.2 ± 0.565	274.0 ± 9.899	555.0 ± 7.071	530.0 ± 5.656	-
Lysine	-	907.0 ± 12.727	30.2 ± 0.5374	483.0 ± 12.727	922.0 ± 11.313	181.0 ± 5.515	469.9 ± 12.162
1-Methyl-Histidine	4.0 ± 0.565	97.5 ± 4.9497	-	267.0 ± 12.727	109.0 ± 7.071	-	57.2 ± 4.808
Arginine	49.7 ± 1.414	482.5 ± 16.404	7.1 ± 0.5656	397.0 ± 7.071	539.5 ± 8.061	397.5 ± 7.071	409.9 ± 14.142

(-) Absent, The values are mean of duplicate determinations with standard deviation (±)

Table 3. Comparison of amino acid content of *Penicillium expansum* with fungal and yeast species.

Amino acids relative %	*Penicillium expansum*	*Candida* LY 496 Bernstein *et al*., 1977	*Leurotus strain* Ginterova & A. Lazarova, 1987	*Aspergillus niger* Christias *et al*., 1975	*Fusarium oxyporum* Christias *et al*., 1975
Phospho-serine	2.05	-	-	-	-
Aspartic Acid	6.60	10.50	11.20	9.00	9.93
Threonine	4.01	5.50	5.16	5.40	5.67
Serine	-	6.10	4.96	6.40	6.01
Glutamic Acid	26.84	16.70	13.70	11.90	12.60
Proline	5.26	-	7.94	5.60	4.77
Glycine	7.94	4.40	3.05	10.10	9.13
Valine	8.88	5.90	5.33	6.50	6.26
Isoleucine	3.80	5.50	4.12	4.30	4.61
Leucine	8.28	8.30	9.39	6.80	7.56
Phenylalanine	4.56	4.80	4.75	3.50	3.25
Alo-Lysine	4.49	-	-	-	-
Halo-lysine	4.50	-	-	-	-
Lysine	7.48	11.80	6.87	6.00	6.31
1-Methyle Histidine	0.88	-	-	-	-
Histidine	-	2.10	4.50	2.00	1.66
Arginine	4.38	5.30	5.29	6.40	3.94
Tyrosine	-	4.20	5.37	2.10	2.37
Methionine	-	1.60	1.26	1.60	1.53
Alanine	-	7.30	7.13	10.00	12.41
Tryptophan	nd	-	-	1.40	1.20
Cystine	nd	-	-	0.90	0.64

Results are given in micromoles per 100mg mycelia (dry weight); nd = Not determined

Acknowledgement

This research project was supported by Pakistan science foundation through its research grant S-SU/Chem-184.

References

Algur O and HZ. Gokalp. 1991a. Some fermentation parameters influencing single cell protein production by *Rhizopus arrhizus* and *Actinomucor elegans*. DOGA. *Tr. J. of Biology*, 15: 190-197.

Algur, O.F. and H.Y. Gokalp. 1991b. Batch cultureof *Rhizophus arrhizus* and *Actinomucor elegans* on vinasse medium for biomass production and BOD reduction. DOGA. *Tr. J. Biol*., 15: 198- 205.

Anderson, R.F. and R.W. Jackson. 1958a. Essential amino acids in microbial protein. *Appl. Microbiol*., 6: 369-373.

Anderson, R.F. R.A. Rhodes, G.E.N. Nelson, M.C. Shekleton, A. Barreto jr. and M. Arnold. 1958b. Lysine, methionine and tryptophan content of microorganisms. *J. Bacteriol*., 76: 131-135.

Anonymous. 1988 Economic Survey Government of Pakistan Finance Division. Economic Advisor's Wing, Islamabad, p. 90.

Bernstein, S., C.H. Tzeng and D.Sisson. 1977. Potential source of energy and chemical products. *Biotechnol. Bioeng. Symp*., 7: 1-9.

Bhattacharjee, J.K. 1970. Microorganisms as potential sources of food. *Adv. Appl. Microbiol*., 13: 139.

Bressani, R., R.G. Brenes, A. Garcie and L.G. Elias. 1987. Chemical composition, amino acid content and protein quality of *Canavalia* spp. seeds. *J. Sci. Food. Agric*., 40: 17.

Burrel, R.G., C.W. Clayton, M.F. Gallegly and V.D. Lilly. 1966. Factors affecting the antigenicity of mycelium of three species of *phytopathorea. Phytopath*., 56: 422-226 .

Christias, C., C. Couvarak, S.G. Georgopoulos, B. Macris and V. Vomvoyanni. 1975. Protein content and amino acids composition of certain Fungi evaluated for microbial protein production. *Appl. Environ. Microbiol*., 29: 250-254.

Crawford, D.L. and E. Mccoy. 1973. Production of microbial protein from waste cellulose by *Thermomonospora fusca*, a thermophilic actnimycate. *Biotechnol. Bioeng*., 15: 833-843.

Daghir, N.J. and T.K. Abdul Baki. 1977. Yeast protein in broiler rations. *Poultry Science*, 56: 836- 841.

Garg, S.K and S. Neelakantan. 1982. Production of SCP and cellulase by *Aspergillus terreus* from bagasse substrate. *Biotechnol. Bioeng*., 24: 109-125.

Garibay, M.G., L.G. Ruiz and E. Barzana. 1987. Studies on the simultaneous production of single cell protein and polygalacturonase from *Kluyveromyces fragilis*. *Biotechnol. Lett*., 9: 411-416.

Ginterova, A. and A. Lazarova. 1987. Amino acid composition of wood rotting fungi (Pleurotus) and total amino acid balance of the cultivating system. *Food Chem*., 23: 35-41.

Gow, J.S., J.D. Littlehoules, S.R.L. Smith and R.B. Walter. 1975. Single cell protein II, (Eds.): S.R. Tanneubaum and D.I.C. Wang. M.I.T. Press Cambridge, pp. 375-384.

Gregory, K.F., A.E. Reade, G.L. Khor, J.C. Alexandar, J.H. Lumsden and G. Losos. 1976. Conversion of carbohydrates to protein by high temperature Fungi. *Food Technol*., 30: 30-35.

Han, Y.W. 1978. Microbial untilization of straw- review. *Adv. Appl. Microbiol*., 23: 119- 149

Harold, V. 1950. *Practical Clinical Biochemistry*, 4th ed. Published by Vazirani for Anrold Heineman Pvt. Ltd. New Dehli India, pp. 195.

Kim, J.H., S. Libuchi and J.M. Lebeault. 1981. Quantitative physiology of *Penicillium cyclopium* grown on whey for production of microbial protein. *Eur. J. Appl. Microbiol. Biotechnol*., 13: 208-212.

Moo-Young, M. 1977. Economics of SCP Production. *Process Biochem*., 12(4): 6-10.

Nelson, G.E.N., R.E. Anderson, R.A. Rhodes, M.C. Shekleton and H.H. Hall. 1960. Lysine, methionine and tryptophan content of microorganism II yeast. *Appl. Microbiol*., 8: 179-182.

Okanishi, M. and K.F. Gregory. 1970. Isolation of mutants of *Candida tropicalis* with increased methionine content. *Can. J. Microbiol*., 16: 1139-1143.

Oloyede, O.B., A.A. Odutuga, J.B. Minari and A.A. Amballi. 2007. Assessment of some serum metabolites and enzymes of broiler-chickens fed raw and processed bambara groundnut. *International Journal of Poultry Science*, 6(9): 647-650.

Onol, A.G. and S. Yalcm. 1995. The usage of baker's yeast in lying hen rations. *Veterinary Journal of Ankara University*, 42: 161-167.

Oshoma, C.E. and M.J. Ikenebomeh. 2005. Production of *Aspergillus niger* biomass from rice bran. *Pakistan Journal of Nutrition*, 4(1): 32-36.

Pacheco, M.T., G.M. Caballero-Cordoba and V.C. Sgarbieri. 1997. Composition and nutritive value of yeast biomass and yeast protein concentrates. *Journal of Nutritional Science and Vitaminology* (Tokyo), 43: 610-612.

Reade, A.E. and K.F. Gregory. 1975. High-temperature production of protein-enriched feed from cassava by fungi. *Appl. Microbiol*., 30(6): 897-904.

Rubbani, B., M. Afzal, M.S. Mubarik, M. Salim and S.M. Hussain. 2011. Estimation of apparent digestibility coefficients of soybean meal based diets with different protein levels for *Labeo rohita. Pak. J. Agri. Sci*., 48: 231-235.

Samuel, B.T. and D.R. Zimmerman. 1977. Evaluation of a yeast single cell protein in Pig diet. *Journal of animal Science*, 45(6): 1309-1316.

Sanna, O. and S. Sabry. 1991. Microbial biomass and protein production from whey. *Journal of Islamic Academy of Sciences*, 4: 170-172.

Scrimshaw, N.S. 1968. Single cell protein. (Eds.): R.I. Mateles and S.R. Tanneubaum. M.I.T. Press Cambridge Mass, pp. 3.

Sehu, A., S. Yalcm and F. Karakas. 1977. The effect of bakers yeast added to the quail rations to growth and carcass yield. *Turkish Journal Veterinary Animal Science*, 21: 221-226.

Yakoub, M. Khan, M. Umar Dahot and M. Yousuf Khan. 1992. Single cell protein production by *Penicillium javanicum* from pretreated rice husk. *J. Islamic Acad of Sciences*, 5: 39-43.

Zabrodskii, A.J. 1972. *Production of fooder yeast from molasses in alcohol factories*. Food Ind. Publ. House Moscow.

(Received for publication 7 November 2010)